PCT/IL2004/000571
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June 04, 2004

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APPLICATION NUMBER: *60/482,437*
FILING DATE: *June 26, 2003*

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PATENT APPLICATION SERIAL NO. _____

U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE
FEE RECORD SHEET

06/27/2003 BSAYASI1 00000034 60482437
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PTO-1556
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PTO/SB/16 (10-01)
Approved for use through10/31/2002. OMB 0651-0032
U.S. Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No.

INVENTOR(S)

Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
MORDECHAI	DEUTSCH	MOSHAV OLESH, ISRAEL
REUVEN	TIROSH	KFAR SABA, ISRAEL

☐ *Additional Inventors are being named on the _____ separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

AN IMPROVED INTERACTIVE TRANSPARENT INDIVIDUAL CELLS BIOCHIP PROCESSOR

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

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☐ Firm or Individual Name	SCHOTTENSTEIN CELLOME RESEARCH CENTER				
Address	BAR-ILAN UNIVERSITY				
Address					
City	RAMAT GAN	State	ISRAEL	ZIP	52900
Country	ISRAEL	Telephone	97235344675	Fax	97235342019

ENCLOSED APPLICATION PARTS *(check all that apply)*

☑ Specification *Number of Pages* 5 ☐ CD(s), Number _____

☑ Drawing(s) *Number of Sheets* 1 ☐ Other (specify) _____

☐ Application Data Sheet. See 37 CFR 1.76

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

☑ Applicant claims small entity status. See 37 CFR 1.27.

☐ A check or money order is enclosed to cover the filing fees

☐ The Commissioner is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: _____

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FILING FEE AMOUNT ($): $80.00

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☑ No.

☐ Yes, the name of the U.S. Government agency and the Government contract number are: _____

Respectfully submitted,

SIGNATURE _____

TYPED or PRINTED NAME MORDECHAI DEUTSCH

TELEPHONE 972 3 534 4675

Date 6/26/2003

REGISTRATION NO. *(if appropriate)* _____

Docket Number: 21

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

This collection of information is required by 37 CFR 1.51. The information is used by the public to file (and by the PTO to process) a provisional application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the complete provisional application to the PTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, Washington, D.C. 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Box Provisional Application, Assistant Commissioner for Patents, Washington, D.C. 20231.

PTO/SB/17 (05-03)
Approved for use through 04/30/2003. OMB 0651-0032
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FEE TRANSMITTAL for FY 2003

Effective 01/01/2003. Patent fees are subject to annual revision.

☑ Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT ($) 80

Complete if Known	
Application Number	
Filing Date	6/26/03
First Named Inventor	MORDECHAI DEUTSCH
Examiner Name	
Art Unit	
Attorney Docket No.	21

METHOD OF PAYMENT *(check all that apply)*

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FEE CALCULATION

1. BASIC FILING FEE

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1001	750	2001	375	Utility filing fee	
1002	330	2002	165	Design filing fee	
1003	520	2003	260	Plant filing fee	
1004	750	2004	375	Reissue filing fee	
1005	160	2005	80	Provisional filing fee	80

SUBTOTAL (1) ($) 80

2. EXTRA CLAIM FEES FOR UTILITY AND REISSUE

	Extra Claims		Fee from below	Fee Paid
Total Claims		-20** =		X =
Independent Claims		- 3** =		X =
Multiple Dependent				=

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description
1202	18	2202	9	Claims in excess of 20
1201	84	2201	42	Independent claims in excess of 3
1203	280	2203	140	Multiple dependent claim, if not paid
1204	84	2204	42	** Reissue independent claims over original patent
1205	18	2205	9	** Reissue claims in excess of 20 and over original patent

SUBTOTAL (2) ($)

**or number previously paid, if greater; For Reissues, see above

FEE CALCULATION (continued)

3. ADDITIONAL FEES

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1051	130	2051	65	Surcharge - late filing fee or oath	
1052	50	2052	25	Surcharge - late provisional filing fee or cover sheet	
1053	130	1053	130	Non-English specification	
1812	2,520	1812	2,520	For filing a request for *ex parte* reexamination	
1804	920*	1804	920*	Requesting publication of SIR prior to Examiner action	
1805	1,840*	1805	1,840*	Requesting publication of SIR after Examiner action	
1251	110	2251	55	Extension for reply within first month	
1252	410	2252	205	Extension for reply within second month	
1253	930	2253	465	Extension for reply within third month	
1254	1,450	2254	725	Extension for reply within fourth month	
1255	1,970	2255	985	Extension for reply within fifth month	
1401	320	2401	160	Notice of Appeal	
1402	320	2402	160	Filing a brief in support of an appeal	
1403	280	2403	140	Request for oral hearing	
1451	1,510	1451	1,510	Petition to institute a public use proceeding	
1452	110	2452	55	Petition to revive - unavoidable	
1453	1,300	2453	650	Petition to revive - unintentional	
1501	1,300	2501	650	Utility issue fee (or reissue)	
1502	470	2502	235	Design issue fee	
1503	630	2503	315	Plant issue fee	
1460	130	1460	130	Petitions to the Commissioner	
1807	50	1807	50	Processing fee under 37 CFR 1.17(q)	
1806	180	1806	180	Submission of Information Disclosure Stmt	
8021	40	8021	40	Recording each patent assignment per property (times number of properties)	
1809	750	2809	375	Filing a submission after final rejection (37 CFR 1.129(a))	
1810	750	2810	375	For each additional invention to be examined (37 CFR 1.129(b))	
1801	750	2801	375	Request for Continued Examination (RCE)	
1802	900	1802	900	Request for expedited examination of a design application	

Other fee (specify) _____

*Reduced by Basic Filing Fee Paid SUBTOTAL (3) ($)

SUBMITTED BY				(Complete *if applicable*)	
Name (Print/Type)	ROBERT VASL	Registration No. (Attorney/Agent)		Telephone	972-3-5344675
Signature				Date	6/26/03

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This collection of information is required by 37 CFR 1.17 and 1.27. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: **Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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PROVISIONAL PATENT APPLICATION

Inventors: MORDECHAI DEUTSCH AND REUVEN TIROSH

Title: AN IMPROVED INTERACTIVE TRANSPARENT INDIVIDUAL CELL S BIOCHIP PROCESSOR

FIELD AND BACKGROUND OF THE INVENTION

The present invention relates to functional cellomics and, more particularly, to a method for the observation and manipulation of individual cells.

Combinatorial (bio)chemistry has evolved as an essential practical means permitting synthesis of many biologically-active and pharmaceutical structures, which must then be tested for their effects on animals and humans. The use of single, individual cell- based assays is an important tool in modern and advanced biomedical studies. Furthermore, cell functions are comprised of many interconnecting signaling and feedback pathways. Many times, a compound study based on isolated targets or cell preparations can not resolve this complexity. Thus, for a comprehensive understanding of a compound effect, testing of a single, whole living cell, is required. Such tests, in addition to their assistance in discovering and developing safer products, provide a useful tool in detecting biological and toxic effects, suggesting an alternative method for present toxicological tests resulted in reducing the number of animals used for testing.

In PCT patent application number WO 03/035824 to Deutsch filed 25 October 2001 there is described an interactive transparent individual cells biochip processor (ITICBP) that allows for the observation and manipulation of single cells in their own individual wells.

There is however a drawback associated with Deutsch's method. In Deutsch's method when cells are observed through a microscope, the material that the wells are made of which is usually glass or plastic-

polystyrene has a significantly different refractive index than the physiological medium that the cells are suspended in. This causes some light scattering. Additionally some of the light will reflect off the glass causing further optical distortion. Additionally in the best of cases, there will be no interaction between the cell and the glass or plastic-polystyrene and in other cases there may be an interfering reaction between the cells and their glass wells.

There is thus a widely recognized need for, and it would be highly advantageous to have, an ITICBP devoid of the above limitations.

BRIEF DESCRIPTION OF THE DRAWING

The invention is herein described, by way of example only, with reference to the accompanying drawing. With specific reference now to the drawing in detail, it is stressed that the particulars shown are by way of example and for purposes of illustrative discussion of the preferred embodiments of the present invention only, and are presented in the cause of providing what is believed to be the most useful and readily understood description of the principles and conceptual aspects of the invention. In this regard, no attempt is made to show structural details of the invention in more detail than is necessary for a fundamental understanding of the invention, the description taken with the drawings making apparent to those skilled in the art how the several forms of the invention may be embodied in practice.

In the drawing:

FIG. 1 is a perspective side view of the present invention.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention is of a hydrogel substitute for a glass grid (matrix) used for the observation and manipulation of individual ordered cells which can be used for overcoming problems relation to glass or plastic poly-styrene grids.

The principles and operation of a hydrogel grid according to the present invention may be better understood with reference to the drawing and accompanying descriptions.

Before explaining at least one embodiment of the invention in detail, it is to be understood that the invention is not limited in its application to

the details of construction and the arrangement of the components set forth in the following description or illustrated in the drawings. The invention is capable of other embodiments or of being practiced or carried out in various ways. Also, it is to be understood that the phraseology and terminology employed herein is for the purpose of description and should not be regarded as limiting.

Referring now to the drawings, Figure 1 illustrates the grid **20** which is part of the whole transparent grid compartment **10.** Transparent grid compartment **10** according to the prior art of Deutsch includes a glass or a plastic polystyrene grid unit **20.** Grid unit **20** may be for example a slab shape of 1 millimeter (mm) thickness and have a frame of 4x4 mm including a centered engraved surface 1x1 mm square grid **21.** Each grid for example may contain up to 50x50 20 μm diameter wells. The size and number of the wells may vary as desired. The depth of the wells may be for example 10 μm. According to the teachings of the present invention a hydrogel grid unit **20** is used to replace the glass grid described in the prior art of Deutsch. The use of a hydrogel instead of a glass or a plastic poly-styrene grid has numerous advantages.

As the refractive index of the hydrogel is much closer to the refractive index of the physiological medium that the cells are in, light scattering from the glass grid and optical distortion will be reduced.

Cells that are settled onto a gel surface will feel a friendlier physiological environment.

Moreover the gels may contain trapped diffusible materials.

In a preferred embodiment that will be described below, an additional thin layer gel cover may be added on top of the cells which would prevent any cell migration between individual wells and additionally do not have any uncontrolled flow of liquid medium between wells, enabling the testing of individual cell uptake and secretion, for example of fluorescent labeled molecules.

Transparent grid compartment **10** further includes a top cover glass **22** and bottom cover glass **23** that press against an inner frame **24** which is preferably made from rubber polymer. Inner frame **24** preferably has at least one inlet and outlet channel **26** configured for cell loading and medium flow, which may be controlled for example by a hydrostatic pressure head. The space **28** above grid **21** is occupied with the transparent medium and enables the microscopic observations of the grid during both cell loading and fluid manipulations.

In order to produce a hydrogel grid unit **20** and to ensure that it will not break up due to its relatively fragile consistency (preferably 96% water), the hydrogel must be secured in its place without any possibility of being moved in relation to its rubber frame. In order to fulfill this purpose, a rubber polymer is poured into space **28** on top of a glass grid **20** which is described above and in the abovementioned prior art of Deutsch. An example of a rubber polymer which may be used is hydrophilic vinil polysiloxane impression material,Injection type,Type 3 Low Viscosity, available as EXAMIX™ NDS, from GC AMERICA INC, ALSIP, IL 60803 U.S.A. At the end of this stage there is now formed a negative template of the glass grid exactly at the correct dimension and position relative to the rubber polymer frame **24**.

Following the rubber polymerization stage, the whole grid compartment **10**, is turned upside down. Cover glass **23** is removed and then glass grid unit **20** is removed. Now a fluid alginate is poured into the space vacated by the removal of glass grid unit **20** and Calcium Gluconate polymeriser is added on top for up to a couple of hours to gelinate. Examples of gels which may be used are thermal gel-sol transition such as agar or gelatin, or room temperature Ca-induced gel of alginate (polysaccharide from seaweed) available as PROTANAL LF120 or LF200, from FMCBioPolymer, P.O.Box 494, N-3002 Drammen, Norway.

Cover glass **23** is then returned to its previous place and then cover glass **22** is removed. Following this, the rubber polymer negative template is extracted and then cover glass **22** is reinstated and the whole transparent grid compartment **10** is secured after grid compartment **10** has been turned around upside down again to its original position.

The hydrogel containing transparent grid compartment **10** is now ready for use.

In practice, cell loading and medium flow through channel **26** may be now performed. The whole setup can be placed under a microscope and cells may be observed with minimal scattering during various fluid manipulations.

The gel may contain various reagents which may interact with the cells that are in the wells formed by the gel.

In addition, a thin layer of gel (not shown) may be poured and polymerized on top of the cells within the same framework. This sandwich type of the planar ordered cells in between two gel films prevents any cell migration as well as identifying an individual

transparent surrounding for each cell. Once this top thin layer of gel is in place various fluid manipulations can be carried out. New reagents can then diffuse through the gel to the cells and from the cells through the gel to the topical flow.

An additional advantage is that fluorescent labeling of the diffusing reagents enables the kinetics of their distribution in the environment of each cell and within the cells themselves.

Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all such alternatives, modifications and variations that fall within the spirit and broad scope of the appended claims.

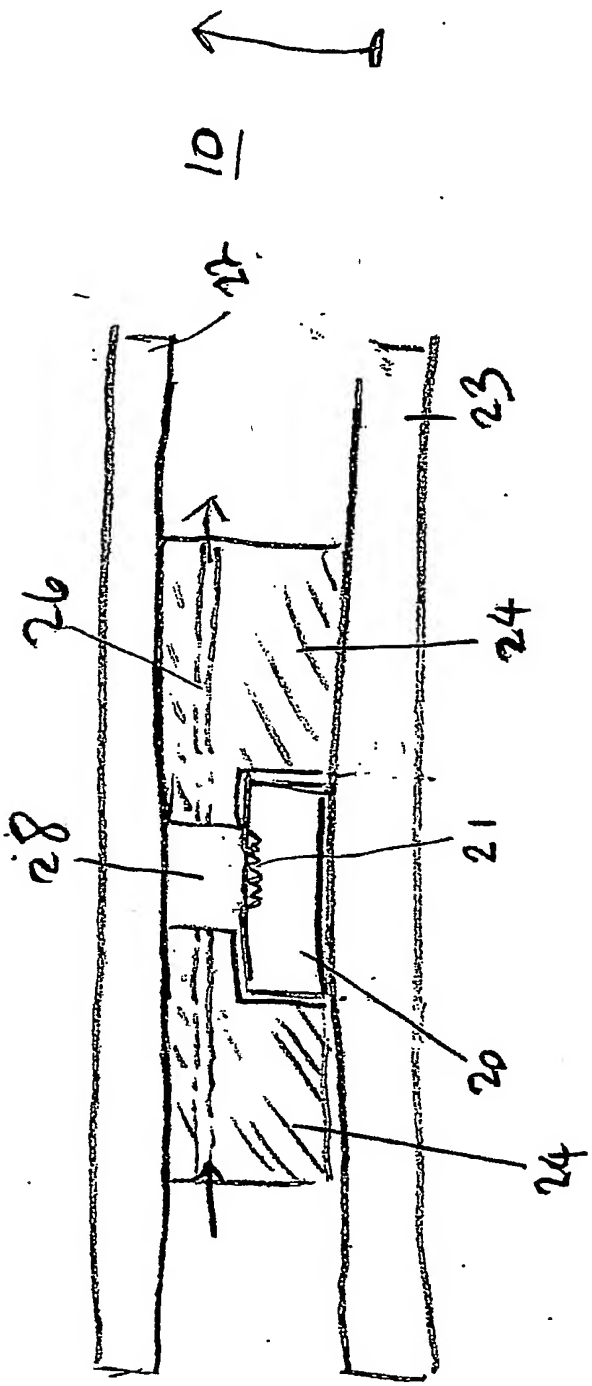

Figure 1